CIGARETTE SMOKE TRACERS: GAS CHROMATOGRAPHIC ANALYSIS OF DECACHLOROBIPHENYL

Summary

Decachlorobiphenyl in microgram quantities was used as a tracer for estimating the deposition of total particulate matter of cigarette smoke in the airways of laboratory animals. This compound, because of its lack of transfer to the pulmonary circulation, is useful as a tracer of total smoke exposure. On the contrary, because of its rapid transfer to the blood, dichlorobenzophenone is best used as a tracer for estimating deposition of smoke in non respiring airways and the study of clearance by mucociliary transport.

Previously, the use of 4,4'-dichlorobenzophenone (DBP) as a tracer for water-insoluble particulate components of tobacco smoke was described.[1] Further studies indicated that the quantity of halogenated tracer found in the respiratory tract of laboratory animals is not only a function of well known clearance mechanisms but also a function of transfer into the blood. It is probable that transfer into the blood can occur at a very rapid rate for many water-insoluble chemical compounds administered by inhalation.

This report presents a sensitive analytic method for decachlorobiphenyl (DCBP) in the animal lung and in the particulate matter of smoke derived from labeled cigarettes. Data are presented for several experiments demonstrating the utility of this tracer. Also, data are presented which indicate that a fraction of the DBP tracer transfers quickly to the blood, whereas DCBP tracer is not transferred in the same time interval.

Data relating to rapid transfer from the lung to the pulmonary circulation of a number of other halogenated compounds are also presented.

Basically, the same methods previously reported[1] for preparing cigarettes, smoking sample cigarettes, exposing animals, and of analysis of tissue, were used in these experiments, except for the conditions of gas chromatography.

For the analysis of DCBP, each tissue sample was homogenized in a semimicro Waring blender jar with 1 ml of a hexane solution containing 1 μg of the internal standard 1,1'-dichlorodiphenyldichloroethane (DDD) per ml. 15 ml of hexane, and 12 g of anhydrous sodium sulfate. The resulting mixture was treated as described previously for tissues containing DBP.

The gas chromatographic conditions for analysis of DCBP were similar to those for analysis of DBP except for the following changes: (*1*) injection port temperature, 285° C; (*2*) column temperature, 260° C; (*3*) electron capture detector temperature, 300° C; (*4*) glass tubing column 1.2 m long by 6 mm in diameter, packed with 3.9 per cent UCW-98 silicone rubber on 80-100 mesh Supelcoport.[2]

The calibration curve for DCBP is shown in figure 1. Recovery of DCBP was 100 per cent when 0.2 to 3.0 μg of DCBP was added to the samples.

Tissue samples containing DDD and 4,4'-dibromodiphenylmethane (DBDM) were analyzed by the previously reported method for DBP[1] using DBP as the internal standard. Calibration curves for each analysis resembled those for DBP.

The data from an experiment with Long-Evans strain rats exposed to eight 40-second puffs of smoke at a rate of one puff per min from DCBP-labeled cigarettes (0.217 per cent DCBP by weight of tobacco, yielding 250 μg of DCBP in the mainstream smoke per cigarette), diluted 6 to 1 with air are shown in table 1 with data from an identical experiment in which DBP cigarettes were used (0.360 per cent DBP by weight of tobacco, yielding 500 μg of DBP in the mainstream smoke per cigarette). In both cases, the lung and trachea of the animals were excised within approximately 2 minutes after exposure. It was found that the lung deposition of DCBP in per cent (13.4 per cent of theoretic) was greater than that for DBP (0.18 per cent of theoretic). Similar results were obtained with hamsters and

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[1] Lewis, C. I., McGeady, J. C., Wagner, J. R., Schultz, F. J., and Spears, A. W.: Amer. Rev. Resp. Dis., 1972, *106*, 480.

[2] Supelco Products, Inc., Bellefonte, Pennsylvania 16823

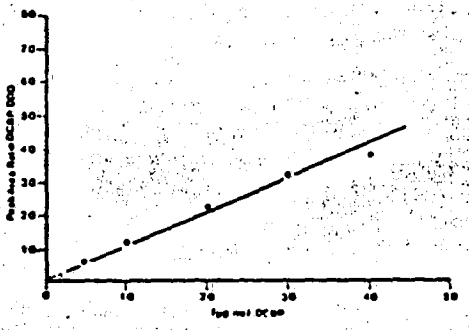

Fig. 1. Calibration curve for analysis of decachlorobiphenyl.

mice. This difference in the fraction of the 2 tracers found in the lung could not be explained by extrapolation of the DCBP tracer clearance curve to zero time as depicted for DBP in the previous study[1]: therefore, it was the result of unusually high disappearance of DBP within 2 minutes after exposure.

To elucidate this rapid clearance, anesthetized, tracheotomized rats were administered labeled smoke generated by a 35-ml puff taken with a syringe in approximately two seconds from the cigarette. By use of a 3-way valve, a 4-ml aliquot of the fifth puff was administered to the animal and a 16-ml aliquot of the sample puff was collected on a Cambridge filter for analysis of the tracer delivered to the animal. After administration of smoke, the trachea was clamped and the lung was excised within 2 minutes. In one type of experiment, laparotomized rats were used in which pneumothorax was produced by a diaphragmatic hernia immediately before administration of smoke. Strong heart beats were maintained during administration of smoke. In another type of experiment, freshly isolated, cannulated, rat lungs were exposed to smoke in a similar manner. The results indicated that the fraction of tracer recovered in a given animal species was a function of molecular weight, and probably molecular size and solubility (table 2). The heaviest (DCBP) and lightest (DBP) mole-

TABLE 2

MEAN RECOVERY OF CIGARETTE SMOKE TRACERS FROM ANESTHETIZED, CANNULATED INTACT RATS WITH PNEUMOTHORAX AND ISOLATED, CANNULATED RAT LUNG-TRACHEA PREPARATION*

Tracer†	Molecular Weight	Lung-Trachea	Recovery
DBP	251	Isolated	83
DBP	251	Intact	11
DDD	320	Intact	40
DBO	326	Intact	64
DCBP	499	Intact	104

*Adult, Long-Evans strain rats. Undiluted smoke (35 ml per 2-second puff) was generated and delivered with a syringe, and the fifth puff was used.

†DBP = 4,4'-dichlorobenzophenone; DDD = 4,4'-dibromodiphenylmethane; DCBP = decachlorobiphenyl; DBO = 4,4'-dibromodiphenylmethane.

TABLE 1

MEAN VALUES OF DEPOSITION OF CIGARETTE SMOKE TRACERS, 4,4'-DICHLOROBENZOPHENONE (DBP) AND DECACHLOROBIPHENYL (DCBP) IN LUNGS OF MALE ANIMALS

Animals*	Tracer	Concentration In Diluted Smoke (μg/ml)	Total Exposure Time (min)	Estimated Ventilation† (ml/min)	Lung Deposition (μg)	Lung Deposition (%)**	DCBP/DBP (%)
Rat (a)	DBP	0.301	5.33	174	1.3	0.48	
Rat (b)	DCBP	0.127	5.33	160	14.5	13.4	28
Hamster (c)	DBP	0.301	5.33	44	0.32	0.46	
Hamster (d)	DCBP	0.127	5.33	70	4.4	9.4	20
Mouse (e)	DBP	0.180	2.40	25	0.05	0.46	
Mouse (f)	DCBP	0.009	2.40	29	0.09	11.1	24

*(a)(b): Ten of 360 g and 4 of 320 g Long-Evans rats per group, respectively.
(c)(d): Twenty of 60 g and 4 of 110 g Golden Syrian per group, respectively.
(e)(f): Twenty of 28 g and 10 of 38 g ICR per group, respectively.

†Calculated according to Guyton.[8]

**Amount of tracer remaining in lung times 100 divided by the amount in inspired air.

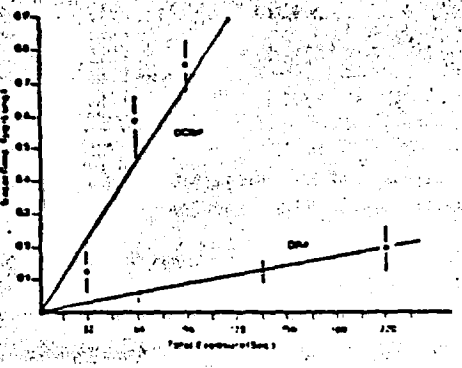

Fig. 2. Concentration of decachlorobiphenyl and 4,4'-dichlorobenzophenone in the lung-tracheal system with increasing smoke exposure time.

cules showed the highest (104 per cent) and lowest (11 per cent) recoveries, respectively. There was a great difference in the per cent recovery of the tracer DBP in the isolated (83 per cent) and intact (11 per cent) lungs (table 2). Because the pulmonary circulation was intact during delivery of noke in the intact preparation, it appeared that the rapid loss of the tracer from the respiratory tract was to the bloodstream. The ratios of deposition of DCBP to deposition of DBP in per cent in lung for the rat, the hamster, and the mouse were 28, 20, and 21 per cent respectively (table 1).

The tissue concentrations of both tracers increased linearly as a function of exposure time (figure 2). Data on pulmonary clearance of both tracers 2 minutes after exposure in a double-labeling experiment in the rat are shown in figure 3 together with clearance data for *E. coli* from the literature.[3]

The fractions of the DBP and DCBP found in the head and stomach-esophagus were of approximately the same order of magnitude, whereas the fractions of DBP and DCBP found in the lung trachea differed more than one order of magnitude (table 3). From these data, and from those presented in figure 2, it was concluded that the DBP that was deposited on inhalation in the respiring area of the lung was transfered to the blood very quickly.

The DCBP found 2 minutes after exposure

[3] Rylander, R.: Acta Physiol. Scand., 1968 (Supplement *306*, p. 77).

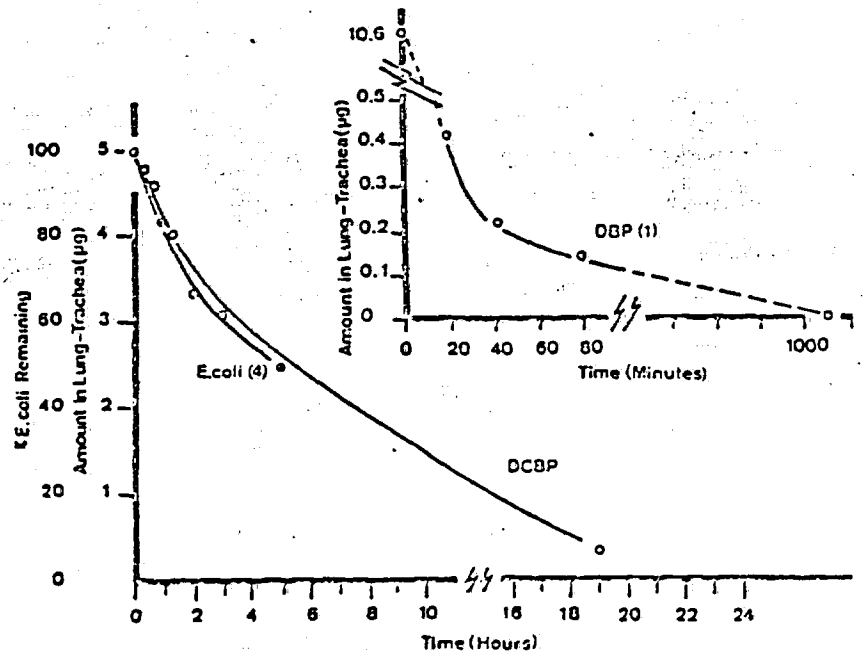

Fig. 3. Rate of clearance of decachlorobiphenyl from the lung tracheal system compared with clearance of *Escherichia coli*[3] and 4,4'-dichlorobenzophenone (DBP).[1]

TABLE 3

DEPOSITION OF CIGARETTE SMOKE TRACERS 4,4'-DICHLOROBENZOPHENONE (DBP) AND DECACHLORODIPHENYL (DCBP) IN MOUSE ORGANS AFTER EXPOSURE TO DOUBLY LABELED SMOKE*

Organ	Tracer†	Conc. in Diluted Smoke (μg/ml)	Total Exposure Time (sec)	Deposition (μg)	Deposition (%)**
Stomach-esophagus	DBP	0.30	144	0.45	2.2
Lung-Trachea	DBP	0.30	144	0.10	0.48
Head††	DBP	0.211	96	0.41	4.2
Stomach-esophagus	DCBP	0.089	144	0.18	2.9
Lung-Trachea	DCBP	0.089	96	0.76	18.4
Head††	DCBP	0.089	96	0.38	9.2

*Mean values of 20 ICR strain male mice (26 g). Ventilation equals 29 ml.[7]

†See table 2 for definitions of abbreviations of compounds.

**Amount of tracer remaining in organ times 100 divided by the amount in inspired air.

††All skin removed.

represents the total quantity deposited in the respiratory tract; the clearance rate was similar to that previously attributed to a combination of mucociliary transport and macrophage activity.[4,5] It is noteworthy that the fraction of *E. coli* (approximately 10 per cent) cleared by mechanical transport was of the same order of magnitude as the fractional ratio of DBP to DCBP found in the respiratory tract 2 minutes after exposure. This is consistent with the suggestion that DBP is recovered only from the nonrespiring portions of the lower respiratory tract.

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Vaughan[6] reported the total retention of radioactive sodium chloride-glycerine aerosols of varying sizes in the lungs of anesthetized rats. For a 0.6-μm aerosol, which is an approximation of the mean particle size for cigarette smoke,[7] the retention was 14 per cent. Using an estimated ventilation of 160 ml per min for the rats (table 1) and the data for the DCBP tracer,[8] the retention of 6:1 diluted smoke was estimated to be 18.4 per cent.

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[6] Vaughan, W. J., and Vaughan, B. E.: USNRDL-TR-68-108, October 4, 1968; National Technical Information Service, Springfield, Virginia.

[7] Leonard, R. E., and Kiefer, J. E.: Tobacco Sci., 1972, *174*, 35.

[8] Guyton, A. C.: Amer. J. Physiol., 1947, *150*, 70.